Thermocycler (Perkin Elmer Cetus, Foster City, CA, USA). The lower PCR reaction mixture (50 µl) contained 5 µl of 10X buffer, 0.4 mM each of four deoxynucleotide triphosphates (Perkin Elmer, Foster City, CA, USA) and 1 to 2 µM each of two primers. The upper PCR reaction mixture (50 µM) contained 5 µl of 10X buffer, 0.5 to 1 µl of Taq Plus or Tsg Plus enzyme, and template DNA. The lower and upper mixtures were separated by a layer of AmpliWax PCR Gem50® (Perkin Elmer, Foster City, CA, USA) before heating cycles started. The thermocycling condition employed for the provision of PCR products in the construction of various plasmids are set forth in Table 11 below. The PCR products were purified using a QIAquick PCR purification kit (Qiagen Inc., Mississauga, Ont., Canada). The purified PCR products were sequenced on both strands directly and/or after cloning in appropriate vectors using an Applied Biosystem sequencer.

Paragraph beginning at line 28 of page 31 has been amended as follows:

The deletion of a short 5' region from the 4223 200 kDa protein gene is shown in Figure 10 and was performed using a similar approach as described in Example 4. An about 500 bp 5' region of the 200 kDa gene was PCR amplified from strain 4223 using primers 5471.KS and 4257.KS (Table 8) from chromosomal DNA. The 5' primer (designated 5471.KS) was based upon the region surrounding the previously identified GTG downstream start codon. In primer 5471.KS, the flanking regions around the GTG codon were incorporated and the GTG was mutated to ATG with further mutations used to introduce an *Nde*I site incorporating the new ATG. Using numbering from the full-length 200 kDa protein, the new start codon would be M56 replacing the previous V56 codon. The 3' primer (designated 4257.KS) was based upon the non-coding strand located about 500 bp downstream from the GTG codon in the 200 kDa protein gene. The PCR-product was digested with *Nde*I, purified using a QIAquick® PCR purification kit (Qiagen Inc., Mississauga, Ont.), and inserted into *Nde*I digested and dephosphorylated pKS122 to provide pKS348 (see Figure 7). Plasmid pKS348 was confirmed by restriction enzyme analyses and by sequencing of the PCR-amplified DNA piece and its joint regions. The nucleotide sequence (SEQ ID No: 12) and the deduced amino acid sequence (SEQ ID No: 13) for the 5'-truncation contained

C3 in pKS348 are shown in Figure 8. A similar N-terminal truncated 200 kDa gene from strain LES-1 was generated in the same manner and was designated pKS444.

Paragraph beginning at line 20 of page 33 has been amended as follows:

E. coli cell pellets were obtained from 500 ml culture prepared as described in Example 7, by centrifugation and were resuspended in 50 ml of 50 mM Tris-HC1, pH 8.0, containing 0.1 M NaCl, and disrupted by sonication. The sonicate was centrifuged at 20,000 xg for 30 min. and the resultant supernatant (sup1) was discarded. The pellet (ppt1) was extracted, in 50 ml of 50 mM Tris-HC1, pH 8.0 containing 0.5% Triton X-100® and 10 mM EDTA, then centrifuged at 20,000 xg for 30 min. and the supernatant (sup2) was discarded. The pellet (ppt2) was further extracted in 50 ml of 50 mM Tris-HC1, pH 8.0, containing 1% octylglucoside, then centrifuged at 20,000 xg for 30 min. and the supernatant (sup3) was discarded.

C4

Paragraph beginning at line 34 of page 33 has been amended as follows:

The resultant pellet (ppt3) contained the inclusion bodies. The pellet was solubilized in 6 ml of 50 mM Tris-HC1, pH 8.0, containing 6 M guanidine and 5 mM DTT. Twelve ml of 50 mM Tris-HCl, pH 8.0 was added, the mixture centrifuged at 20,000 xg for 30 min, and the pellet (ppt4) discarded. The supernatant (sup4) was precipitated by adding polyethylene glycol (PEG) 4000 at a final concentration of 5% and incubated at 4°C for 30 min. The resultant pellet (ppt5) was removed by centrifugation at 20,000 xg for 30 min. The supernatant was then precipitated by $(NH_4)_2SO_4$ at 50% saturation at 4°C overnight. After the addition of $(NH_4)_2SO_4$, the solution underwent phase separation with protein going to the upper phase (as judged by the cloudiness of the layer). The upper phase was collected, then subjected to centrifugation at 20,000 xg for 30 min. The resultant pellet was collected and dissolved in 2 ml of 50 mM Tris-HCl, pH 8.0, containing 6 M guanidine and 5 mM DTT. The clear solution was purified on a Superdex 200® gel filtration column equilibrated in 50 mM Tris-HC1, pH 8.0, containing 2 M guanidine HC1. The fractions were analysed by SDS-PAGE and those containing the purified r200 kDa were pooled. The pooled

C5

C5 fraction was concentrated 5 to 10 fold using a centriprep 30 and then dialysed overnight at 4°C against PBS, and centrifuged at 20,000 xg for 30 min to clarify.

Paragraph beginning at line 20 of page 37 has been amended as follows:

C6 Twenty-five µl of diluted pre-immune serum or test antiserum were added to the wells of a 96-well Nunclon® microtitre plate (Nunc, Roskilde, Denmark). Twenty-five µl of diluted bacterial cells were added to each of the wells. A guinea pig complement (BioWhittaker, Walkerville, MD) was diluted 1:10 in VBS, and 25 µl portions were added to each well. The plates were incubated for 60 min, gently shaking at 70 rpm on a rotary platform. Fifty µl of each reaction mixture were plated onto Mueller Hinton agar plates (Becton-Dickinson, Cockeysville, MD). The plates were incubated at 37°C for 24 hours, and then left at room temperature for a further 24 hours. The number of colonies per plate was counted, and average values of colonies per plate were estimated from duplicate pairs.

In the Claims:

Claims 11 to 23 have been cancelled.

Claims 1 and 3 have been amended as follows:

Sub D1 C7 1. (Amended) An isolated and purified nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence consisting of SEQ ID Nos: 5, 6, 8 or 10 or the complementary sequence thereto,

(b) a nucleotide sequence encoding an about 200 kDa outer membrane protein of a strain of *Moraxella catarrhalis* and consisting of SEQ ID Nos: 7, 9 or 11, and

(c) a nucleotide sequence encoding an about 200 kDa outer membrane protein of another strain of *Moraxella catarrhalis* which is characterized by a tract of consecutive G nucleotides which is 3 or a multiple thereof in length, an ATG start codon about 80 to 90 bp upstream of said tract and said tract being

C7 located between about amino acids 25 and 35 encoded by the nucleotide sequence.

3. (Amended) An isolated and purified nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence consisting of SEQ ID No: 12,

(b) a nucleotide sequence encoding the derived amino acid sequence consisting of SEQ ID No: 13, and

C8 (c) a nucleotide sequence encoding a 5'-truncation of a gene encoding an about 200 kDa outer membrane protein of another strain of *Moraxella catarrhalis* and which expresses the corresponding N-terminally truncated about 200 kDa outer membrane protein from *E. coli*.